Assessing Stress Responses in Beaked and Sperm Whales in the Bahamas

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LONG-TERM GOALS

The long-term goal of this project is to develop fecal hormone assays to assess stress responses in Blainville's beaked whales (*Mesoplodon densirostris*) and sperm whales (*Physeter macrocephalus*) inhabiting the northern Bahamas. These deep-diving species were chosen to include a particularly acoustically-sensitive cetacean (beaked whales) and a co-occurring species (sperm whales) for comparison. The immediate goals are to: 1) evaluate the feasibility of fecal sample collection from these two species, 2) validate immunoassays and determine fecal hormone levels for relatively undisturbed reference populations of both species off Great Abaco Island in the Bahamas, and 3) characterize the natural variations in stress-related hormones according to life history stage (age-class, sex, reproductive status). The results of this project will provide baseline levels of these hormones in beaked and sperm whales for comparison with conspecifics inhabiting environments with acoustic exposures, such as the nearby U.S. Navy Atlantic Undersea Test and Evaluation Center (AUTEC), and experiencing known acoustic disturbances including mid-frequency active sonar.

OBJECTIVES

The objectives of the research project in FY2015 were to:

(1) Complete dedicated fecal sampling surveys for Blainville's beaked whales and sperm whales off southwest Great Abaco Island. Fieldwork not completed in previous years (due to poor weather conditions and whale abundance shifts) was extended into FY2015.

(2) Process and analyze fecal samples collected in FY2014 for five steroid and thyroid hormones at the NEAq Marine Endocrinology Laboratory.

(3) Conduct preliminary hormone data analyses.

APPROACH

This project is a collaboration between scientists at the John H. Prescott Marine Laboratory at the New England Aquarium (NEAq; Boston, MA) and the Bahamas Marine Mammal Research Organisation (BMMRO; Great Abaco Island, The Bahamas). Sample collection is being led by BMMRO scientists (D. Claridge, C. Dunn) with assistance from NEAq scientists (R. Rolland, S. Kraus, K. Hunt, E. Burgess). Initial sample processing is being conducted in a field laboratory equipped with a centrifuge and basic equipment at BMMRO in the Bahamas. BMMRO is providing individual identification and life history information on sampled whales. Endocrine analyses, data interpretation, project oversight and management, and reporting are being conducted by the NEAq (R. Rolland, K. Hunt, S. Kraus).

BMMRO conducted dedicated fieldwork for this project during August and September 2015. Fecal samples were collected from Blainville's beaked whales and sperm whales using methods previously employed by BMMRO with modifications made by NEAq scientists (see Rolland 2011, 2013). Sample collection was accompanied by photo-identification when possible and photographs of whales that were sampled were later compared to existing identification catalogues to provide information on the individual's sex and age-class.

Fecal samples collected in FY2014 (n = 15) were analysed in FY2015 for five different hormone metabolites (estrogens, progestins, androgens, glucocorticoids, and thyroid hormone) using methods previously validated and described (Rolland et al. 2012). The results were added to the project hormone databases, and preliminary data analyses were conducted.

WORK COMPLETED

Task 1. Field Effort in the Bahamas
Dedicated sampling surveys were conducted by BMMRO during 13 vessel-days in August and September 2015 using a 6.8m rigid-hulled inflatable boat. The success of beaked whale sampling, in particular, is dependent upon a Beaufort seastate ≤ 2 and good underwater visibility, and, therefore, days at sea were chosen carefully based on optimal conditions. During December 2014 and June – July 2015, the field team collected sperm whale samples while working on other projects for an additional 3 days at no added cost to the project. Because on some days survey time was interrupted by increased wind conditions, total dedicated effort consisted of 11.5 days (plus 2.5 leveraged days). Dr. Elizabeth Burgess (NEAq post-doc) assisted BMMRO with fieldwork in The Bahamas from August 23-Sept. 15, 2015.

Surveys were primarily concentrated near the 1000 m isobath along the southwest side of Great Abaco Island, in the northern Bahamas (Fig. 1). Survey effort totaled 1186 km of vessel track lines (235 km were from leveraged days). Sample collection methods have been previously reported in detail (Rolland 2011, 2013). The collection method for beaked whale feces used a small dip net and a one gallon plastic zip-type bag. Sperm whale feces were collected by scooping the feces directly into a Falcon tube or other plastic sample container. Samples were stored in a cooler on ice-packs while at sea, then processed and stored in a standard freezer at the BMMRO field station.

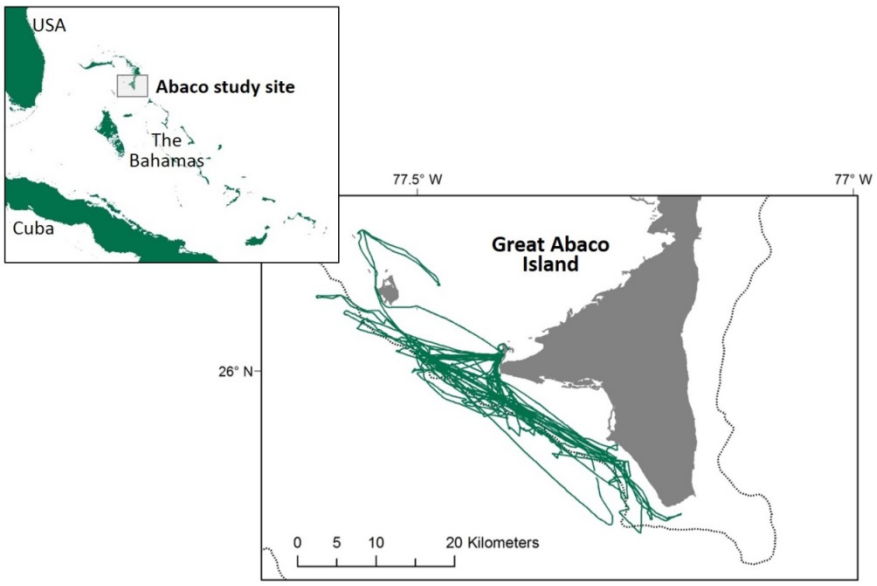

Figure 1. Vessel tracks (green lines) during dedicated and leveraged surveys off the coast of Great Abaco Island between December 2014 and September 2015. Survey efforts were concentrated off the southwestern side of the island along the 1000 m isobaths (gray dashed line).

Task 2a. Hormone Assay Validations
This task was completed in FY2012 with development of fecal sample processing and hormone extraction protocols for both species, and successful validation of immunoassays for fecal estrogens, progestins, androgens, glucocorticoids and thyroid hormones in both species (Rolland et al. 2011).

Task 2b. Hormone Assays
Analysis of all samples collected in FY2014 was completed in the current year of the project. Samples collected in FY2015 will be shipped to the NEAq in November 2015 for hormone analysis.

Task 3. Data Analysis, Publications and Reporting
We completed preliminary analysis of hormone results from FY2011-2014.The majority of statistical analyses will take place after the results from the samples collected FY2015 are available. The FY2014 Annual Report to ONR was submitted. At least one publication is planned after a complete hormone data set is available including results from samples collected during FY2015.

RESULTS

There were 24 cetacean sightings during surveys (including five different species), which comprised 6 groups of Blainville's beaked whales and 7 groups of sperm whales. Group size ranged from 2-7 whales for Blainville's beaked whales (mean = 5) and 1-12 animals for sperm whales (mean = 5).

Fecal Sample Collection
All fecal samples collected for this project were located off the southwest side of Great Abaco Island (Fig. 2). Additionally, three sperm whale samples were collected opportunistically in the vicinity of the AUTEC range off of Andros Island.

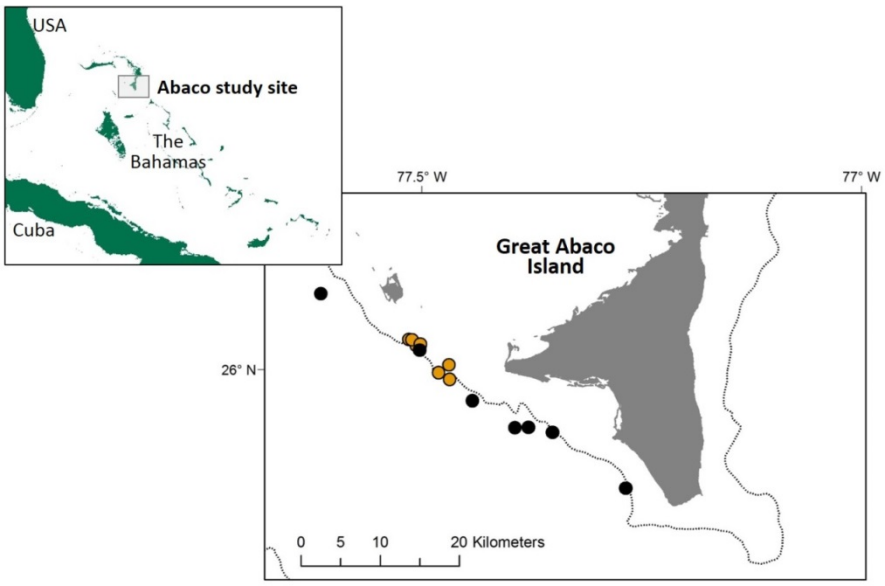

Figure 2. Locations of fecal sample collections during surveys off Great Abaco Island between August and September 2015. Blainville's beaked whale sample locations are represented by orange circles and sperm whales by black circles. The 1000m isobath is shown (gray dashed line).

Fifteen fecal samples were successfully collected from Blainville's beaked whales (*n* = 8) and sperm whales (*n* = 7) during the field effort (Table 1). Up to five samples were collected from beaked whales in a single survey day. Control water samples were also collected in the vicinity of whales (n = 3), and will be tested for immunoreactivity in the assays (e.g. background hormone-like activity in seawater). Information on the age-class, sex and reproductive status based on individual life histories for identified whales are summarized in Table 1. All beaked whale samples except one were linked to known whales; however, only three of the seven sperm whale samples were matched to individuals in the photo-identification catalogue.

Samples were collected from both the mother and calf for two mother-calf beaked whale pairs (Md094 and calf, and Md134 and calf). Both females were presumably lactating during the field collection period because both calves are less than one year old. Md094 was first seen in 1998 and this is her third recorded calf. Md134 was first seen in 1999 as a calf, and had her first calf in 2011, and this is her second recorded calf. Additionally, Md135's calf (Md298) was sampled repeatedly (*n* = 3) over 17 days. Md135 was first seen in 1999 as a calf and had her first calf in 2008. Md298 is Md135's second recorded calf and was first seen in 2011.

Three sperm whales that were sampled have been seen in previous years. Pm019 was first seen in SW Abaco in 1997 and is presumed to be an adult female based on size, summer-time occurrence and long-term site fidelity. Pm043 was first seen in SW Abaco in 1999 and is a confirmed female from biopsy sample analysis. Pm169 was first seen in SW Abaco in 2009 and is also a confirmed female through biopsy sample analysis.

Table 1. Summary of life history data for whales sampled in FY2015.

INDIVIDUAL LIFE HISTORY DATA				
Sample #	**Whale ID**	**Sex***	**Age class**	**Reproductive State**
Blainville's beaked whales				
Md 15-01	Md298 (calf of Md135)	Male	Calf	Immature
Md 15-02	calf of Md094	Male	Calf	Immature
Md 15-03	Md298 (calf of Md135)		Calf	Immature
Md 15-04	Md094	Female	Adult	Mature with calf
Md 15-05	possibly calf of Md134?			
Md 15-06	calf of Md134	Female	Calf	Immature
Md 15-07	Md134	Female	Adult	Mature with calf
Md 15-08	Md298 (calf of Md135)		Calf	Immature
Sperm whales				
Pm14-16	Unknown whale		Subadult	
Pm14-17	Pm169	Female	Adult	Mature with calf
Pm15-01	Pm043	Female	Adult	
Pm15-02	Unknown whale		Subadult	
Pm15-03	Pm019	Female	Adult	
Pm15-04	Unknown whale		Adult female / subadult male	
Pm15-05	Unknown whale			

*If ID is unknown, age and sex class information is from visual observations in the field.

Including all fieldwork years, total fecal samples collected for this project is 68 including 28 samples from beaked whales and 40 samples from sperm whales (Table 2). The two 2010 sperm whale samples were collected opportunistically by BMMRO and were contributed to this project.

Table 2. Fecal samples collected for this project from Blainville's beaked whales and sperm whales.

Year	Beaked Whales	Sperm Whales	Total
2010		2	2
2011	10	9	19
2013	9	6	15
2014	1	16	17
2015	8	7	15
TOTAL	**28**	**40**	**68**

Reproductive Hormone Results

Fecal samples of adequate mass had measurable levels of most of the five hormones in beaked whales. Only one beaked whale sample was collected in 2014 from an adult male; however, the sample mass was extremely small (0.0068g dry weight), and below the threshold for reliable hormone results (< 0.02g; Hayward et al. 2010). Of the 20 samples analyzed to date, results from 12 samples of adequate mass to yield reliable hormone results were summarized (1 sample from a calf, and 1 juvenile sample with incomplete hormone results were not included). Preliminary data analyses for samples collected in FY2011-2014 showed expected patterns in most reproductive hormones based on life history stage of identified whales (Table 3). For example, reproductive hormones in female beaked whales (n=8) varied according to reproductive state, with mature females showing higher progestins and total estrogens compared to juvenile females. Although the sample size is small (n = 2) mean androgen

concentration in adult males is higher than in all female groups. Therefore, biologically valid patterns are present in the reproductive hormones.

The range of glucocorticoid (GC) levels (8.7- 37.4 ng/g) was tighter than thyroid hormones (6.6-150.8 ng/g), and adult males tended to have higher GCs compared to other groups as has been described for other cetaceans (Hunt et al. 2006). One adult male and one adult female had highly elevated T3 levels (143.3 ng/g, 150.8 ng/g) compared to other whales and also had the highest total estrogens in the data set (180.3 ng/g, 121.5 ng/g). This outlying value for thyroid led to apparent higher mean thyroid hormone in adult males because of the small sample size. Statistical comparisons will be conducted when the full hormone data set is available.

Table 3. Fecal hormone results (ng/g) for beaked whales (mean ± SEM). Sample sizes are in parentheses. Adult females are unaccompanied by calves.

	Progestins	**Estrogens**	**Androgens**	**Glucocorticoids**	**Thyroid**
Females (8)					
Juvenile (2)	33.6 ± 0.18	27.6 ± 16.6	13.2 ± 6.6	21.6 ± 6.4	29.5 ± 0.9
Lactating (3)	39.5 ± 15.5	27.5 ± 22.6	5.9 ± 3.1	21.5 ± 8.4	24.9 ± 11.4
Adult (3)	51.6 ± 7.7	76.2 ± 52.4	2.9 ± 1.7	15.4 ± 6.8	17.5 ± 6.8
Males (2)					
Adult (2)	45.6 ± 26.5	64.4 ± 57.1	28.3 ± 22.7	27.2 ± 8.5	81.2 ± 69.6
Unknown sex (2)					
Juvenile(2)	43.2 ±1.4	28.0 ± 18.5	7.8 ± 1.6	14.9 ± 4.7	16.5 ± 4.4

Thirty sperm whales samples (of 33 analysed) had adequate sample mass and produced results for all five hormones. Of these, 19 samples were from identified whales and the hormone results were summarized (Table 4). A subadult male sperm whale (Pm 156) was repeatedly sampled (n=7) over a one month period. The coefficient of variation for all five hormones in the samples from Pm 156 ranged from 7-14%, and the average CV for all hormones was a very respectable 11%. There were 16 samples from whales of known sex, and thus far no samples have been collected from adult male sperm whales. Adult females showed the highest progestin levels, although total estrogens did not follow this pattern. Fecal progestins in both sexes and all age-classes were present at higher concentrations than other hormones. Because of the large proportion of unknown sex and /or age-class whales in this data set, a classification tree analysis is an approach that will be explored when the full data set is available. In contrast to beaked whales, thyroid hormones had a relatively tight range of values (3.7-30.3 ng/g) compared to GCs (37.0-199.7 ng/g). The highest levels of GCs were in adult females and juvenile males. Statistical comparisons will be conducted when the full hormone data set is available.

Table 4. Fecal hormone (ng/g) results for sperm whales (mean ± SEM). Sample sizes are in parentheses.(* = single values)

	Progestins	Estrogens	Androgens	Glucocorticoids	Thyroid
Females (5)					
Juvenile (1)*	6,858.5	153.0	71.0	41.1	17.5
Adult (3)	20,496.3 ± 6,009.7	69.0 ± 7.4	126.8 ± 34.7	131.8 ± 50.5	11.5 ± 2.5
Unknown (1)*	6,872.0	130.7	74.9	33.2	15.9
Males (11)					
Juveniles (11)	13,283.1 ± 1695.6	104.1 ± 18.2	120.4 ± 14.0	136.9 ± 16.7	9.3 ± 0.7
Unknown sex (14)					
Juvenile (5)	6,590.0 ±578.6	145.3 ± 40.0	110.6 ± 20.1	94.0 ± 26.8	16.5 ± 4.0
Adult (1)*	14,484.2	131.5	165.7	49.6	17.5
Unknown (8)	10,200.0 ± 3,090.7	174.4 ± 48.3	107.4 ± 28.3	71.7 ± 15.4	10.9 ± 2.1

IMPACT/APPLICATIONS

Developing methods to better understand the sub-lethal, physiologic consequences of underwater noise disturbance on species of concern, like beaked whales, is crucial to evaluate the potential for long-term impacts of naval exercises and other oceanic activities. The results of this research project have shown that it is feasible to collect fecal samples from both species, and that analysis of stress-related fecal hormones can be successfully applied in both beaked and sperm whales. Although collecting samples from beaked whales, in particular, is challenging, baseline ranges of stress-related fecal hormones are being developed and can be applied in the future to assess physiologic responses to elevated acoustic exposures from naval activities.

RELATED PROJECTS

The NEAq's Marine Endocrinology Program includes a related ONR-funded project on *Development of Novel Noninvasive Methods of Stress Assessment in Baleen Whales* (K. Hunt, PI; ONR #N000141310639). This research project is developing the use of fecal aldosterone assays as an additional measure of adrenal activation during stress responses in North Atlantic right whales (*Eubalaena glacialis*), and is exploring the feasibility of measuring stress-related hormones in respiratory vapor from the same species.

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